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Assessment of Ambient Ozone Effects on Vegetation Using Snap Bean as a Bioindicator Species

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ABSTRACT

Tropospheric ozone is an air pollutant that is toxic to plants, causing visible injury to foliage and a reduction in growth and yield. The use of plant bioindicators is one approach to assess the ozone impacts in diverse geographical areas. The objective of this study was to evaluate snap bean (*Phaseolus vulgaris* L.) as a potential bioindicator species. Three snap bean genotypes known to exhibit a range of ozone sensitivity were grown in pots under charcoal-filtered (CF) or nonfiltered (NF) treatments in open-top chambers, or under ambient air (AA) conditions. Treatment effects on biomass were not significant at 56 days after planting (DAP), but midseason foliar injury increased in the NF and AA treatments relative to CF controls. An increase in ozone from 25 to 30 nL L^{-1} in CF controls to approximately 50 nL L^{-1} in the NF and AA treatments was found to suppress final pod dry weight per plant by 40 to 60% in the most sensitive genotype S156. The same treatments suppressed final pod dry weight by 20 to 30% in a moderately sensitive genotype Oregon-91, and by 10% or less in a tolerant genotype R123. An S156 to R123 yield ratio of approximately one was observed under CF conditions. The S156 to R123 yield ratio declined to 0.6 to 0.7 in the NF treatment and declined further to 0.4 to 0.5 in the AA treatment, suggesting that ozone impact was underestimated in the open-top chambers. The results suggest that a snap bean bioindicator system has the potential to detect ambient ozone effects at present-day ozone concentrations.

Plants are sensitive to tropospheric ozone (Heagle, 1989; Krupa et al., 2001; Morgan et al., 2003), but the ozone response can be quite variable depending on the species and environmental factors (Heagle, 1989). A number of plant species are known to develop visible injury symptoms associated with ozone exposure (Krupa et al., 2001). However, visible injury does not always translate into yield loss (Heagle and Letchworth, 1982). To address this issue, a white clover (*Trifolium repens* L.) bioindicator system has been developed that relates biomass loss to ambient ozone concentrations (Heagle et al., 1995). The clover system is based on ozone-sensitive and -resistant clones that produce similar forage biomass under low ozone conditions, but are differentially affected at elevated ozone concentrations typical of present day pollution levels. The clones are maintained by vegetative propagation and are currently used in a number of research programs in the United States and Europe.

Snap bean is known to be an ozone-sensitive species (Krupa et al., 2001). A genetic cross between an ozone-sensitive cultivar (Oregon-91) and ozone-insensitive cultivar (Wade) resulted in a population that exhibited a wide range of ozone sensitivity (Reinert and Eason, 2000). From this population, snap bean lines have been developed that exhibit a range of ozone response in terms of biomass production under elevated ozone conditions (Burkey and Eason, 2002). The relative ozone sensitivity of one line, S156, appeared to be much greater than the sensitive parent Oregon-91, and thus might be capable of detecting effects at ambient ozone concentrations typically found in areas that are subject to repeated air pollution events during the growing season. The objective of this study was to determine the effects of ambient ozone on the growth and yield of S156, the ozone-sensitive parent Oregon-91, and a tolerant line R123 as a first step in assessing the potential for developing a snap bean bioindicator system that could serve as an alternative to the clover system.

MATERIALS AND METHODS

Plant Culture

The study was conducted for two years (2000 and 2001) at our field site located 5 km south of Raleigh, NC. Seeds of R123 (ozone-tolerant), Oregon-91 (moderately ozone-sensitive), and S156 (ozone-sensitive) genotypes of snap bean were planted directly into 15-L pots of moistened Metro Mix-200 (Scotts-Sierra Horticultural Products, Marysville, OH). Pots were placed in open-top field chambers (Heagle et al., 1973) under charcoal-filtered (CF) or nonfiltered (NF) air conditions, or in chamber frames without panels that served as ambient air (AA) plots. Pot temperatures were moderated with an insulating cylinder composed of 0.6-cm-thick bubble wrap coated on both sides with aluminum (Reflectix, Markleville, IN) fit tightly around each pot. Plants were irrigated with drip tubes on days when the predicted maximum temperature exceeded 32°C, or as needed to prevent visible symptoms of water stress.

In 2000, seeds were planted on 15 May (8 pots per genotype per plot for a total of 24 pots per plot) and thinned to 1 plant per pot at 16 days after planting (DAP). Plants were fertilized with 1 L of Peter's 20–10–20 water-soluble nutrient solution (Scotts-Sierra Horticultural Products) at 10 DAP (0.2 g L^{-1}), 15 DAP (0.6 g L^{-1}), and then weekly (1.6 g L^{-1}). One liter of 0.25 g L^{-1} Peter's standard trace element mixture (Scotts-Sierra Horticultural Products) was applied once at 22 DAP. Thrips were controlled with acephate (Orthene 7S at 4 mL L^{-1}; Valent USA, Walnut Creek, CA) on 26 May and again on 6 June.

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Abbreviations: AA, ambient air; CF, charcoal filtered; DAP, days after planting, NF, nonfiltered; PAR, photosynthetically active radiation.

In 2001, seeds were planted on 22 May (6 pots per genotype per plot for a total of 18 pots per plot) and thinned to 1 plant per pot at 16 DAP. Nutrition was provided by 60 g of Osmocote (14–14–14, N–P–K) slow release fertilizer (Scotts-Sierra Horticultural Products) incorporated into each pot before planting. One liter of 0.25 g L^{-1} Peter's standard trace element mixture was applied once at 17 DAP. Thrips were controlled with acephate (Orthene 7S at 4 mL L^{-1}) on 6 June. Root rot was identified in certain plants on 29 June, and all pots were immediately treated with Ridomil Gold (0.0085 mL L^{-1}; Novartis Crop Protection, Greensboro, NC) to prevent further spread of Phytophthera and Pythium. Spider mites were controlled with abamectin (Avid 0.15 EC at 0.33 mL L^{-1}; Novartis Crop Protection) on 6 August.

Experimental Treatments

The treatment design was a 3 × 3 factorial with three ozone treatments (CF, NF, or AA) and three snap bean genotypes (Oregon-91, R123, and S156). The experimental design was a randomized complete block of four replicates with open-top chambers (or chamber frames in the case of the AA treatment) serving as main plots and the three genotypes as subplots. The three genotypes were randomly assigned to the first three pot locations in the northwest corner of each plot, establishing a plant order that was repeated as a serpentine pattern throughout the plot. Ozone was monitored in each plot using a UV photometric ozone analyzer (Thermo Environmental Instruments, Franklin, MA).

Measurements

Ambient temperature, humidity, and photosynthetically active radiation (PAR) were recorded on site throughout the experiment. Three-minute data averages were recorded for 23.5 h per day. Temperature averages were calculated over the entire daily period while vapor pressures were calculated and averaged only during daylight.

In 2000, a midseason biomass analysis was conducted at 56 DAP. Three plants per genotype from the south half of each plot were harvested and separated into stems, leaves, filled pods (pods with obvious seed expansion), and immature pods (small pods with no obvious seed expansion). Roots were washed to remove growth media components. Separated plant parts were dried at 55°C and weighed. Treatment of the remaining five plants per genotype in each plot continued until a majority of the pods were brown. Pods were separated into mature pods that contained at least one seed or small sterile pods. Pods were counted, dried at 55°C, and weighed.

In 2001, the midseason biomass harvest was replaced with a nondestructive assessment of foliar injury, which was conducted at 57 DAP. This change in protocol was introduced because significant effects of ambient ozone on biomass were not observed at this developmental stage in 2000 and because the nondestructive measurements provided greater numbers of plants for final yield assessment. Upper canopy injury was estimated in 5% increments (0–100%) on two plants of each genotype in all plots. Experimental treatments continued until a majority of the pods were brown. Pods were separated into mature pods that contained at least one seed or small sterile pods. Pods were counted, dried at 55°C, and weighed. The number of plants harvested per genotype varied from three to six in each plot because plants showing apparent root rot symptoms at 38 DAP were not included in the data set.

Statistics

Because there was a one-week difference between planting dates between 2000 and 2001, comparisons of meteorological data between the two years were based on weeks after planting rather than actual dates. Comparisons were made on the values for total daily PAR (mol m^{-2} d^{-1}), mean daylight vapor pressure (kPa), and mean daily temperature (°C). Means of data for each week were compared by analysis of variance.

Plant data were analyzed as plot means determined from two to six plants depending on the variable and year. Residual plots were examined to identify variables that required transformation. All variables were analyzed without transformation except for final harvest mature pod number and sterile pod weight that were analyzed with the square root transformation. Data were analyzed using general linear models.

RESULTS

Final Yield

Season-long studies were conducted with plants subjected to ambient and subambient ozone conditions using open-top chambers. Charcoal filters were used to create a subambient CF control treatment with ozone levels reduced approximately 40% relative to NF and AA treatments in both years of the study (Table 1).

Yield was assessed as pod dry weight at the end of the season. Two types of pods were harvested. Large mature pods containing at least one seed accounted for 94 to 99% of final yield. The remainder of the pod yield was consisted of small sterile pods where growth was arrested early in development. Genotype differences in yield potential were assessed under CF conditions where pod dry weight per plant was greatest. For both years, yield was similar in R123 and S156, but was attained through a different combination of factors. Genotype R123 developed fewer mature pods than S156, but the mass per mature pod was greater for R123 (Table 2). Yield was slightly higher for Oregon-91 compared with R123 and S156 as the result of a greater number of mature pods in the high mass category.

Ozone treatment had a significant effect on pod yield

Table 1. Ozone concentrations.

Treatment	Year	Ozone†				
		25–31 May	1–30 June	1–31 July	1–21 August	Seasonal mean
		nL L^{-1}				
Charcoal-filtered	2000	30	33	32	30	31
	2001	34	25	21	26	25
Nonfiltered	2000	47	53	51	48	51
	2001	56	48	41	46	46
Ambient air	2000	45	52	49	47	49
	2001	56	49	42	49	47

† Twelve-hour mean measured 0800 to 2000 h EST.

(Table 2). Ambient ozone levels (see Table 1) of approximately 50 nL L^{-1} in the NF and AA treatments were associated with a decrease in pod yield relative to CF controls where ozone levels were 25 to 30 nL L^{-1}. A significant genotype × treatment interaction was observed that reflected genotype differences in ozone sensitivity. Ambient ozone in the NF and AA treatments levels had a minimal effect on the yield of the R123, the most tolerant genotype examined in this study. In contrast, yield was reduced in the sensitive genotypes by 20 to 30% in Oregon-91 and by 40 to 60% in S156 (calculated from data in Table 2).

A significant year × treatment interaction was observed for final pod yield. Although NF and AA ozone levels were similar in both years (Table 1), the ozone impact on pod dry weight was greater in 2001 than in 2000. A 15 to 20% increase in productivity of CF controls in 2001 and a greater fractional loss under NF and AA conditions contributed to this effect. A comparison of environmental factors between years revealed only a few significant differences (Fig. 1). Mean daily temperature was different during Weeks 2, 4, and 5 after planting, Weeks 4 and 5 coinciding with rapid floral development. However, during Week 4, temperature was higher in 2001 and during Week 5 the opposite was true. The large difference in temperature at Week 10 occurred after pod development was well advanced. The only important difference in PAR occurred during Week 6 in the early pod development stage. Further, the average daylight vapor pressure was only different during Week 4 after planting in a direction that would suggest a higher level of evaporative stress during that period in 2000. Overall, differences in meteorological conditions did not appear to explain the differences in yield response between years. Alternatively, the different fertilization regimes in 2000 and 2001 could have caused differences in nutritional status of the plants that may explain the year × treatment interaction. The use of a slow release fertilizer in 2001 could have provided a more consistent nutrient supply that resulted in plants with greater yield potential under CF conditions and a greater susceptibility to ozone in the NF and AA treatments.

Two subtle differences in plant response were observed when chambers and ambient air plots were compared. First, ozone impact on pod yield appeared to be underestimated in NF chambers relative to AA plots even though ozone levels in the two treatments were essentially identical. For Oregon-91, NF pod yield was greater than AA in 2000 but similar to AA in 2001, a contributing factor in the year × treatment interaction (Table 2). For S156, pod yield was slightly higher under NF conditions relative to AA in both years. Second, the number of small sterile pods on R123 plants was greater in CF and NF chambers relative to AA plots. This phenomenon occurred in both years and appeared to be a unique characteristic of R123. The basis for these chamber effects presumably involved environmental factors, perhaps the slightly elevated air temperatures associ-

Table 2. Effects of ambient ozone on snap bean final harvest parameters. Values are experiment means (standard error) of four replicate plots each of the charcoal-filtered (CF), nonfiltered (NF), and ambient air (AA) treatments.

Genotype	Treatment	Sterile pod number	Sterile pod weight	Mature pod number	Mature pod weight	Average mature pod weight	Total pod yield
		plant^{-1}	g dry wt. plant^{-1}	plant^{-1}	g dry wt. plant^{-1}	g dry wt. pod^{-1}	g dry wt. plant^{-1}
				2000			
R123	CF	61.0 (3.8)	2.11 (0.19)	67.9 (3.9)	62.5 (5.4)	0.91 (0.05)	64.6 (5.5)
	NF	80.7 (14.0)	2.80 (0.41)	72.4 (5.9)	74.1 (8.4)	1.03 (0.04)	76.9 (8.8)
	AA	11.7 (3.1)	0.46 (0.15)	67.2 (5.2)	79.3 (8.9)	1.16 (0.04)	79.8 (8.8)
Oregon-91	CF	15.7 (0.7)	0.86 (0.04)	72.1 (3.3)	70.3 (3.5)	0.96 (0.01)	71.2 (3.4)
	NF	14.6 (1.3)	0.54 (0.05)	73.1 (1.9)	72.0 (5.9)	0.98 (0.06)	72.5 (5.8)
	AA	13.3 (1.5)	0.52 (0.12)	54.0 (5.2)	53.3 (5.8)	0.97 (0.02)	53.8 (5.9)
S156	CF	23.6 (3.3)	0.84 (0.19)	77.6 (2.1)	60.6 (1.2)	0.78 (0.03)	61.4 (1.1)
	NF	15.2 (2.4)	0.42 (0.08)	69.3 (5.9)	47.9 (4.3)	0.68 (0.01)	48.3 (4.3)
	AA	20.6 (4.1)	0.75 (0.13)	50.9 (3.7)	37.0 (2.1)	0.71 (0.04)	37.7 (2.2)
				2001			
R123	CF	73.5 (8.8)	3.30 (0.45)	75.8 (2.4)	72.0 (3.2)	0.94 (0.02)	75.3 (3.5)
	NF	80.0 (10.9)	3.83 (0.60)	70.5 (2.3)	63.5 (4.0)	0.91 (0.03)	67.3 (3.5)
	AA	6.4 (0.8)	0.36 (0.11)	51.0 (2.6)	70.3 (4.8)	1.36 (0.09)	70.7 (4.7)
Oregon-91	CF	26.8 (2.7)	1.41 (0.13)	88.5 (5.1)	82.0 (4.0)	0.93 (0.02)	83.4 (4.1)
	NF	29.3 (3.8)	1.63 (0.29)	69.5 (3.5)	59.2 (2.9)	0.85 (0.04)	60.8 (3.1)
	AA	7.9 (1.2)	0.36 (0.03)	55.3 (3.0)	59.6 (2.9)	1.08 (0.02)	60.0 (2.9)
S156	CF	15.5 (3.2)	0.53 (0.11)	91.2 (6.9)	72.5 (6.5)	0.80 (0.02)	73.0 (6.4)
	NF	23.1 (7.3)	0.63 (0.16)	61.1 (6.4)	39.3 (3.4)	0.65 (0.02)	39.9 (3.5)
	AA	11.0 (3.6)	0.47 (0.14)	38.1 (2.8)	29.8 (3.0)	0.77 (0.05)	30.2 (3.1)
Source	df			*P* > *F*			
Year†	1	0.517	0.101	0.649	0.572	0.575	0.723
Treatment‡	2	<0.0001	<0.0001	<0.0001	0.006	<0.0001	0.039
Year × treatment‡	2	0.056	0.0002	0.014	0.043	0.0005	0.044
Genotype§	2	<0.0001	<0.0001	0.071	<0.0001	<0.0001	<0.0001
Year × genotype§	2	0.325	0.018	0.106	0.601	0.594	0.612
Treatment × genotype§	4	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
Year × treatment × genotype§	4	0.457	0.418	0.335	0.563	0.395	0.592

† Error term = block(year), df = 6.
‡ Error term = block × treatment(year), df = 12.
§ Error term = block × genotype(year × treatment), df = 24.

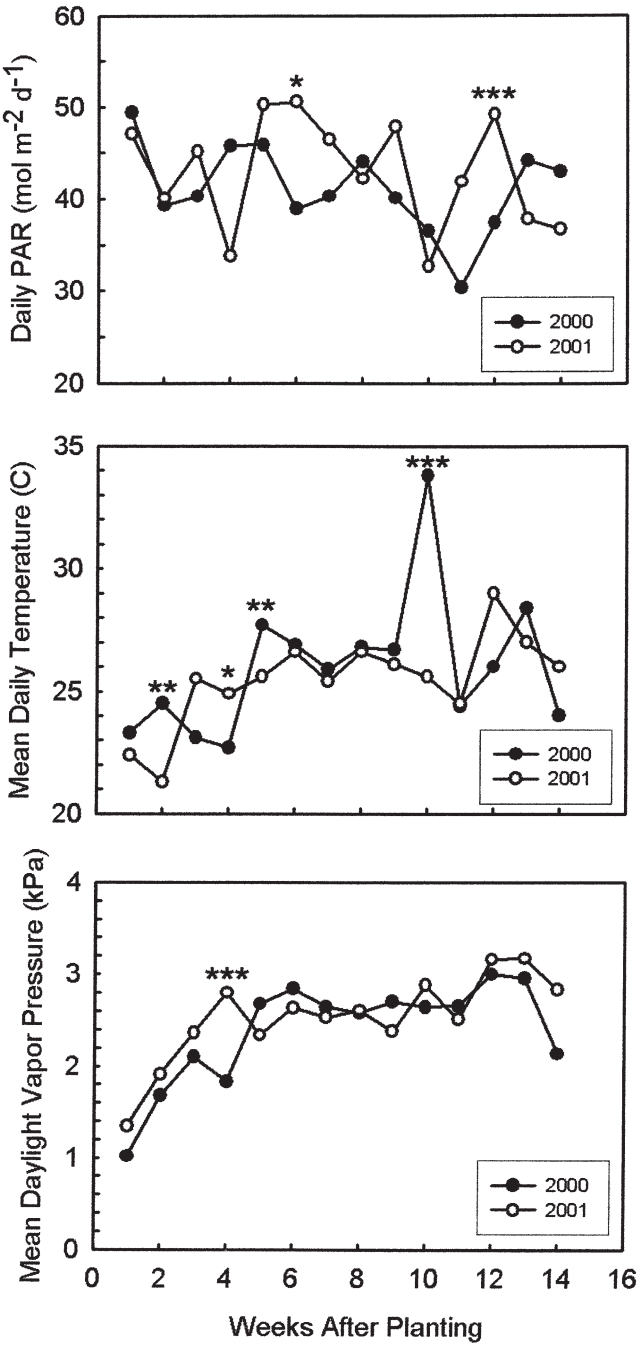

Fig. 1. Meteorological conditions during 2000 (closed circles) and 2001 (open circles) snap bean experiments. Daily photosynthetically active radiation (PAR), mean daily temperature, and mean daylight vapor pressure are compared on the basis of weeks after planting with a planting date of May 15 in 2000 and May 22 in 2001. Significant differences between years within a given weekly period are indicated at the 0.05 (*), 0.01 (**), and 0.001 (***) probability levels.

ated with open-top chamber systems (Heagle et al., 1973).

Midseason Assessments

During the 2000 experiment, a complete midseason biomass analysis was performed at 56 DAP. Significant differences between the three genotypes were found that reflected differences in development. Both R123 and Oregon-91 produced greater total biomass than S156 (Table 3). Further examination of biomass partitioning revealed distinct patterns for each of the three genotypes. The leaf, stem, and root fractions were all greater for R123 (Table 3), evidence that R123 produced larger plants than Oregon-91 or S156. Large pod dry weight was greatest for Oregon-91 at this point in the season. A comparison of midseason (Table 3) and final harvest (Table 2) data averaged across treatments showed that Oregon-91 had accumulated approximately 73% of final pod dry weight at this point in the season. By comparison, S156 had accumulated 66% of final yield and R123 only 40%. Genotype R123 was unique in that pod development was delayed in the CF and NF treatments relative to AA, another example of a chamber effect on the reproductive biology of this genotype.

Although genotype differences were significant at the 2000 midseason harvest, the cumulative ozone impact was not sufficient to produce a significant treatment effect on biomass. At 56 DAP, no treatment effect on total plant dry weight or large pod dry weight was found (Table 3). Apparently, ozone levels of approximately 50 nL L^{-1} in the NF and AA treatments were not sufficient to significantly suppress biomass at this point in the season. Previous studies have shown that somewhat higher ozone levels of approximately 70 nL L^{-1} can suppress the midseason biomass of Oregon-91 and S156, but not R123 (Burkey and Eason, 2002).

During the 2001 experiment, significant genotype and treatment effects were found when foliar injury was assessed at 57 DAP (Table 3). The injury pattern followed the predicted ozone sensitivity of the genotypes. Injury was greatest for S156, intermediate for Oregon-91, and lowest for R123. A significant ozone treatment effect was found for the sensitive genotypes Oregon-91 and S156 with greater foliar injury observed in both the NF and AA treatments relative to CF controls. However, injury was more severe in AA plots relative to NF chambers, further evidence that the open-top chambers tended to reduce the impact of ambient ozone in this study.

DISCUSSION

Using an ozone-sensitive snap bean genotype grown in pots under CF conditions as a reference, this study demonstrated that an ambient ozone seasonal mean of 50 nL L^{-1} was sufficient to suppress pod yield on the order of 50%. The effect was less dramatic for more tolerant genotypes, illustrating that genetics plays a critical role in determining ozone impact. The results support previous observations that differences in ozone sensitivity exist between and within plant species (Guzy and Heath, 1993; Wellburn and Wellburn, 1996). The implication for crops is that diversity within the available germplasm may be sufficient to develop ozone-tolerant cultivars for food and fiber production. Efforts to identify and manipulate ozone tolerance mechanisms at the cellular and molecular levels may one day provide the

Table 3. Effects of ambient ozone on snap bean midseason parameters. Values are experiment means (standard error) of four replicate plots each of the charcoal-filtered (CF), nonfiltered (NF), and ambient air (AA) treatments.

		2000						2001
Genotype	Treatment	Small pod weight	Large pod weight	Leaf weight	Stem weight	Root weight	Total biomass	Visible injury
		g dry wt. plant^{-1}						%
R123	CF	2.83 (1.32)	23.7 (1.9)	27.1 (2.4)	39.8 (3.2)	12.0 (1.4)	105.4 (7.8)	11 (3)
	NF	4.72 (0.65)	21.8 (3.3)	29.6 (2.5)	45.7 (3.4)	12.4 (1.1)	114.1 (9.6)	11 (2)
	AA	1.18 (0.16)	42.2 (6.4)	22.1 (2.3)	34.8 (4.4)	9.8 (1.7)	110.0 (13.6)	25 (3)
Oregon-91	CF	0.41 (0.06)	47.1 (5.5)	13.8 (0.5)	22.3 (1.1)	10.2 (0.6)	93.8 (6.3)	15 (4)
	NF	0.70 (0.23)	50.7 (4.3)	14.5 (1.9)	23.5 (1.3)	8.8 (1.4)	98.2 (8.7)	28 (4)
	AA	1.06 (0.15)	43.6 (2.2)	12.0 (1.2)	19.0 (1.4)	8.9 (1.8)	84.5 (5.8)	44 (3)
S156	CF	0.57 (0.04)	31.0 (9.0)	11.6 (1.1)	17.2 (2.4)	7.6 (1.0)	68.1 (12.7)	23 (6)
	NF	1.18 (0.16)	31.6 (2.0)	11.6 (0.9)	22.4 (2.2)	7.1 (1.1)	73.9 (4.7)	42 (9)
	AA	1.33 (0.12)	30.3 (2.8)	7.5 (0.4)	15.9 (1.1)	6.0 (1.0)	61.0 (3.9)	63 (6)
Source	df	$P > F$						
Treatment†	2	0.053	0.642	0.112	0.087	0.609	0.638	0.0005
Genotype‡	2	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment × genotype‡	4	0.012	0.017	0.541	0.674	0.678	0.882	0.146

† Error term = block × treatment, df = 6.
‡ Error term = block × (treatment × genotype), df = 12.

basis for additional increases in the ozone tolerance of cultivated plants. However, the implications are quite different for natural ecosystems where genetic manipulation is neither practical nor logical. Current ambient ozone levels are sufficient to cause visible injury on plants in natural ecosystems including tall milkweed (Chappelka et al., 1997), black cherry (Chappelka et al., 1999), and native wildflowers (Chappelka et al., 2003), but the long-term impact is not known at present. There is a growing concern that increasing ambient ozone levels will alter competition between sensitive and tolerant species within a plant community resulting in a negative impact on biodiversity (Krupa et al., 2001).

In several key aspects, the ozone response observed in this study resembled the clover bioindicator system developed by Heagle et al. (1995). The ozone-sensitive (NC-S) and resistant (NC-R) clover clones produce similar amounts of biomass under low-ozone conditions resulting in a NC-S to NC-R ratio of approximately one (Heagle et al., 1991). Similarly, snap bean genotypes evaluated in this study generated final harvest pod dry weight ratios in the range of 0.9 to 1.1 under CF conditions (Table 4). For the clover clones, the NC-S to NC-R biomass ratio declined as ambient ozone levels increased (Heagle et al., 1995), reflecting greater suppression of NC-S growth in polluted environments. A similar response was observed in this study for snap bean. An analysis of the three possible sensitive–tolerant genotype pairings (S156 with R123, S156 with Oregon-91, and Oregon-91 with R123) showed that final pod dry weight ratios were reduced in both NF and AA treatments relative to CF controls in all cases (Table 4). The treatment effect was greatest for the S156–R123 pair that represented the extremes of ozone sensitivity used in this study. The decline in S156 to R123 yield ratio from approximately 1.0 at 30 nL L^{-1} ozone (CF seasonal mean) to approximately 0.5 at 50 nL L^{-1} ozone (AA seasonal mean) suggested that a snap bean system has the potential to detect ambient ozone effects at present-day ozone concentrations.

Additional testing will be required to determine whether the results reported here can be developed into a snap bean bioindicator system analogous to the clover system. An assessment can be made of the S156 and R123 snap bean lines as a potential sensitive and tolerant genotype pair in the proposed bioindicator system. Advantages of the S156–R123 pair include a common genetic background (derived from the same parents), similar pod yield under low ozone conditions, and large differences in foliar injury and pod yield under elevated

Table 4. Final harvest yield ratios. Values are experiment means (standard error) of four replicate plots each of the charcoal-filtered (CF), nonfiltered (NF), and ambient air (AA) treatments.

		Yield ratio		
Year	Treatment	S156 to R123	S156 to Oregon-91	Oregon-91 to R123
2000	CF	0.97 (0.07)	0.87 (0.04)	1.12 (0.08)
	NF	0.68 (0.14)	0.68 (0.08)	0.97 (0.09)
	AA	0.48 (0.03)	0.72 (0.06)	0.68 (0.08)
2001	CF	0.98 (0.11)	0.89 (0.13)	1.12 (0.08)
	NF	0.60 (0.04)	0.68 (0.08)	0.90 (0.05)
	AA	0.44 (0.06)	0.51 (0.07)	0.85 (0.03)
Source	df	$P > F$		
Year†	1	0.574	0.171	0.501
Treatment‡	2	0.0003	0.036	0.002
Year × treatment‡	2	0.893	0.394	0.304

† Error term = block(year), df = 6.
‡ Error term = block × treatment(year), df = 12.

ozone conditions. Genotype S156 appears to be an extremely sensitive genotype with pod yield suppressed by 50% at 50 nL L^{-1} ozone (this study) and up to 90% at 72 nL L^{-1} ozone (Heagle et al., 2002). These characteristics are important factors in the selection of plant material to be used as ozone bioindicator plants. However, S156 and R123 exhibit subtle but potentially important differences in growth. Genotype R123 is a somewhat larger plant (greater biomass) and has larger pods than S156, although pod dry weight per plant at the final harvest is similar under low ozone conditions. In addition, R123 produced a greater number of small sterile pods than S156 when grown in open-top chambers, possibly reflecting a subtle genotype difference in reproductive biology. Efforts are underway to determine whether other lines from the original Reinert and Eason (2000) snap bean cross exhibit the same ozone-tolerance as R123 with a growth habit more similar to S156. This will be the next step in the development of a potential snap bean bioindicator system for assessment of ambient ozone effects on vegetation. A snap bean system would have the advantage over the clover system in that production and storage of seeds would be an easier method of propagation than maintaining virus-free plants for vegetative cuttings. However, multiple planting dates would be required to obtain a series of snap bean harvests during a growing season that are currently available from a single clover planting.

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